

Claims

1. A RP-II protease selected from the group consisting of:
- 5 (i) a RP-II protease that is immunochemically identical or partially identical by cross-reaction with an antibody raised against or reactive with at least one epitope of a RP-II protease comprising the amino acid sequences of the mature peptides shown in the appended Sequence Listing SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12; and/or
- 10 (ii) a RP-II protease that is at least 60% homologous with the amino acid sequence of a RP-II protease comprising the amino acid sequence shown in the appended Sequence Listing SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12; and/or
- 15 (iiia) a RP-II protease that is encoded by a DNA sequence which hybridizes with an oligonucleotide probe hybridizing with a DNA sequence encoding a RP-II protease comprising the amino acid sequence shown in the appended Sequence Listing SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12; and/or
- 20 (iiib) a RP-II protease that is encoded by a DNA sequence which hybridizes with an oligonucleotide probe hybridizing with a DNA sequence encoding a RP-II protease comprising the DNA sequence shown in the appended Sequence Listing SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, or SEQ ID NO: 11;
- 25 an allelic variant of (i), (ii), or (iiia or iiib); a subsequence of (i), (ii), (iiia or iiib), or (iv), wherein the subsequence has protease activity.
- 30
- 35

2. A RP-II protease variant, wherein at least one Asn and/or Gly in an Asn-Gly sequence has been modified by substitution, deletion and/or insertion to change or remove said Asn-Gly sequence.
- 5
3. The protease variant of claim 2, wherein the parent protease is selected from the group consisting of BLC, AC116, CDJ-31, BO32, JA96, AA513, MPR or variants thereof.
- 10 4. A RP-II protease variant, wherein any Glu and/or Asp residue has been modified by substitution or deletion.
- 15 5. The protease variant of claim 4, wherein the parent protease is selected from the group consisting of BLC, AC116, CDJ-31, BO32, JA96, AA513, MPR or variants thereof.
- 20 6. A RP-II protease variant wherein the amino acid residue occupying the 1st and/or 2nd position following a Glu or Asp has been modified by replacing said Glu or Asp residue with a Pro residue.
- 25 7. The protease variant of claim 6, wherein the parent protease is selected from the group consisting of BLC, AC116, CDJ-31, BO32, JA96, AA513, MPR or variants thereof.
- 30 8. A RP-II protease variant wherein at least one amino acid residue susceptible to oxidation exposed to the surface of the molecule, is deleted or replaced with another amino acid residue less susceptible to oxidation.
- 35 9. The variant of claim 8, wherein the amino acid residue susceptible to oxidation is methionine.
10. The variant of claim 9 comprising a replacement by an acid residue selected from the group consisting of A, E, N, Q, I, L, S and K.
11. The protease variant of any of claims 8, 9, or 10, wherein the parent protease is selected from the group

consisting of BLC, AC116, CDJ-31, BO32, JA96, AA513, MPR or variants thereof.

12. A RP-II protease variant wherein one or more tryptophan residues at the surface of the protease has been deleted or substituted.

13. The variant of claim 12 comprising the modification of said tryptophan residues by substitution with F, T, Q or G.

10 14. The protease variant of any of claims 12, or 13, wherein the parent protease is selected from the group consisting of BLC, AC116, CDJ-31, BO32, JA96, AA513, MPR or variants thereof.

15 15. A RP-II protease variant wherein one or more tyrosine residues at the surface of the protease has been substituted.

20 16. The variant of claim 15 comprising the substitution of said Tyr with Phe or Trp.

25 17. The protease variant of any of claims 15, or 16, wherein the parent protease is selected from the group consisting of BLC, AC116, CDJ-31, BO32, JA96, AA513, MPR or variants thereof.

18. A RP-II protease BLC variant comprising E152{R,K,G}.

30 19. A RP-II protease variant essentially as specifically disclosed herein.

20. An isolated polynucleotide having a nucleic acid sequence encoding a RP-II protease, selected from the group consisting of:

35 (a) a nucleic acid sequence encoding a RP-II protease having an amino acid sequence which has an identity of at least 60% with an amino acid sequence of the mature polypeptide of SEQ ID NO: 2, 4, 6, 8, 10 or 12;

- (b) a nucleic acid sequence having at least 60% homology with a nucleic acid sequence encoding a mature RP-II protease of SEQ ID NO: 1, 3, 5, 7, 9, or 11;
- 5 (c) a nucleic acid sequence which hybridizes under low stringency conditions with (i) the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, or 11, (ii) the cDNA sequence of SEQ ID NO: 1, 3, 5, 7, 9, or 11, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii);
- 10 (d) an allelic variant of (a), (b), or (c); and/or
- 15 (e) a subsequence of (a), (b), (c), or (d), wherein the subsequence encodes a RP-II protease fragment which has protease activity.
- 20 21. The polynucleotide of claim 20, which encodes a RP-II protease having an amino acid sequence which has at least 60, 70, 80, 90, or 95% identity with the amino acid sequence of the mature peptide of SEQ ID NO: 2, 4, 6, 8, 10, 12, or 14, or corresponding subsequences thereof.
- 25 22. The polynucleotide of claim 20, which encodes a RP-II protease comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 12.
- 30 23. The polynucleotide of claim 20, which encodes a RP-II protease consisting of the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10 or 12, or a fragment thereof which has protease activity.
- 35 24. The polynucleotide of claim 28, which encodes a RP-II protease consisting of the amino acid sequence of the mature peptide of SEQ ID NO: 2, 4, 6, 8, 10 or 12

- 25 The polynucleotide of claim 20, which has at least 60, 70, 80, 90, or 95% homology with the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, or 11.
- 5 26. The polynucleotide of claim 20, which has the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, or 11.
- 10 27. The polynucleotide of claim 20, wherein the polynucleotide hybridizes under low, medium or high stringency conditions with (i) a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, or 11, (ii) a polynucleotide comprising the cDNA sequence of SEQ ID NO: 1, 3, 5, 7, 9, or 11, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand 15 of (i), (ii), or (iii).
- 20 28. The polynucleotide of claim 20, which is contained in the plasmid pUC19/AC116; pUC/CDJ31; pUC/BO32; pUC/JA96; or pUC/AA513 which is contained in DSM 12841: *E. coli* pUC19/AC116; DSM 12842: *E. coli* pUC/CDJ31; DSM 12843: *E. coli* pUC/BO32; DSM 12844: *E. coli* pUC/JA96; or DSM 12845: *E. coli* pUC/AA513.
- 25 29. The polynucleotide of any of claims 20-28, which encodes a RP-II protease that has at least 20% of the protease activity of the RP-II protease of SEQ ID NO: 2, 4, 6, 8, 10, or 12.
- 30 30- An isolated polynucleotide comprising a nucleic acid sequence having at least one mutation in the mature RP-II protease coding sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13, in which the mutant nucleic acid sequence encodes a mature polypeptide of SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14.
- 35 31. An isolated polynucleotide produced by (a) hybridizing a DNA under low, medium or high stringency conditions with (i) the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13, (ii) the cDNA sequence of SEQ ID NO: 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or

(iv) a complementary strand of (i), (ii), or (iii); and (b) isolating the polynucleotide.

32. A nucleic acid construct comprising the polynucleotide
5 of any of claims 20-31 operably linked to one or more control sequences that direct the production of the RP-II protease in a suitable expression host.

33. A recombinant expression vector comprising the nucleic acid construct of claim 32, a promoter, and transcriptional and translational stop signals.

34. A recombinant host cell comprising the nucleic acid construct of claim 33.

15 35. A method for producing a mutant polynucleotide, comprising (a) introducing at least one mutation into the mature polypeptide coding sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13, wherein the mutant polynucleotide encodes a RP-II protease consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14; and (b) recovering the mutant polynucleotide.

36. A mutant polynucleotide produced by the method of claim 35.

25 37. A method for producing a RP-II protease, comprising (i) cultivating a strain comprising the mutant polynucleotide of claim 36 encoding the RP-II protease to produce a supernatant comprising the RP-II protease; and (ii) recovering the RP-II protease.

30 38. A method for producing a RP-II protease comprising (i) cultivating the host cell of claim 34 under conditions suitable for production of the RP-II protease; and (ii) recovering the RP-II protease.

39. A method for producing a RP-II protease comprising

SEARCHED INDEXED COPIED SERIALIZED FILED

- (i) cultivating a host cell under conditions conducive for production of the RP-II protease, wherein the host cell comprises the mutant polynucleotide of claim 36 encoding the RP-II protease to produce a supernatant comprising the polypeptide; and
5 (ii) recovering the RP-II protease.

40. A nucleic acid construct comprising a gene encoding a protein operably linked to one or both of a first 10 polynucleotide encoding a signal peptide from SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13 and a second polynucleotide encoding a propeptide from SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13, wherein the gene is foreign to the first and second polynucleotides.

15 41. A recombinant expression vector comprising the nucleic acid construct of claim 40.

or 42. A recombinant host cell comprising the nucleic acid construct of claim 40 or the expression vector of claim 41.

20 43. A method for producing a protein comprising
(i) cultivating the recombinant host cell of claim 42 under conditions suitable for production of the protein; and
25 (ii) recovering the protein.

44. A detergent composition comprising a RP-II protease of any of claims 1 to 19.

30 45. The composition of claim 44 comprising at least one further enzyme, preferably another protease, preferably a subtilisin of the subtilase group I-S2 or high alkaline subtilisins, preferably subtilisin PB92, subtilisin 309, subtilisin 147, or alkaline elastase YaB or functional variants thereof.

35 46. The compositions of claim 44, wherein the other enzyme is a lipase, cellulase, amylase, peroxidase or oxidase.

47. A method of using a RP-II protease of any of the claims
1 ~~to~~ in a detergent composition.

Add'l